

Impact of signal peptide and transmembrane segments on expression and biochemical properties of a lipase from *Bacillus sphaericus* 205y

ABSTRACT

A total of 97 amino acids, considered as the signal peptide and transmembrane segments were removed from 205y lipase gene using polymerase chain reaction technique that abolished the low activity of this enzyme. The mature enzyme was expressed in *Escherichia coli* using pBAD expression vector, which gave up to a 13-fold increase in lipase activity. The mature 205y lipase (without signal peptide and transmembrane; –SP/TM) was purified to homogeneity using the isoelectric focusing technique with 53% recovery. Removing of the signal peptide and transmembrane segments had resulted in the shift of optimal pH, an increase in optimal temperature and tolerance towards more water-miscible organic solvents as compared to the characteristics of open reading frame (ORF) of 205y lipase. Also, in the presence of 1 mM inhibitors, less decrease in the activity of mature 205y lipase was observed compared to the ORF of the enzyme. Protein structure modeling showed that 205y lipase consisted of an α/β hydrolase fold without lid domain. However, the transmembrane segment could effect on the enzyme activity by covering the active site or aggregation the protein.

Keyword: *Bacillus sphaericus* 205y; Thermostable lipase; Signal peptide; Transmembrane; Purification; Improve properties